Exhibit B

NOTEBOOK NO. 2233

ISSUED TO R. Saiki

ON _____, _____ 19____

DEPARTMENT 113

RETURNED _____ 19____

SCIENTIFIC NOTEBOOK CO.
5007 WEST DONNA DRIVE
STEVENSVILLE, MICHIGAN 49127

Obtained ~100μl of second prep Taq polymerase from David (heparin-Sepharose, ~~frac~~ frac 52-53, concentrated)

20 mM Tris-Cl pH 8
0.1 M KCl
0.1 mM EDTA
1 mM DTT
50% Glycerol
} storage buffer (note: no gelatin)

7400–11,700 u/ml
0.1 M Salmon Sperm DNA.

Variation in concentration reflects results obtained in two ~~dif~~ assays using different dNTP stocks. Higher value (12 u/μl) with assay components used in assay of ~~first~~ first batch Taq polymerase (2167:65).

If variation in u/μl depending on DNA substrate is same as before, concentration with m13 primer extension is 21 u/μl!

This stuff is ~3x more concentrated than first lot.

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(lot 2)

Assay Taq polymerase 1 for activity in PCR amplification of β-globin using Molt4 and PC03/04. Vary # cycles (15-30) and amt. enzyme used (1-4 μl). Also include lot 1 polymerase as control.

15° 20° 25° 30° ~ # cycles

A B C D:	4 μl	lot 1	25 μl per sample containing 250 ng genomic DNA
E F G H:	1	lot 2	
I J K L:	2	"	
M N O P:	4	"	

Molt4 @ 100 μg/ml, PC03 and PC04 @ 10 μM, dNTP @ 40 mM

50 μl	DNA (5 μg)	Split into four 100 μl samples. Heat → 20', 98° Cool to room temp and add amounts of enzyme indicated above. each Sub-divide further into four 25 μl samples and subject to indicated number of cycles. (After overlaying with mineral oil.)
50 μl	10x salts	
50 μl	2 mg/ml gelatin	
50 μl	PC03	
50 μl	PC04	
50 μl	DMSO "	
75 μl	dNTP	
125 μl	H_2O	
500 μl		

program: 2½' @ 98°
5' @ 37°

A, E, I, M → 14 cycles	After last cycle, heat 10-15' @ 60° to finish last ~~extend~~ extension.
B, F, J, N → 19 "	
C, G, K, O → 24 "	
D, H, L, P → 29 "	

Extract mineral oil with $CHCl_3$ and store at -20°

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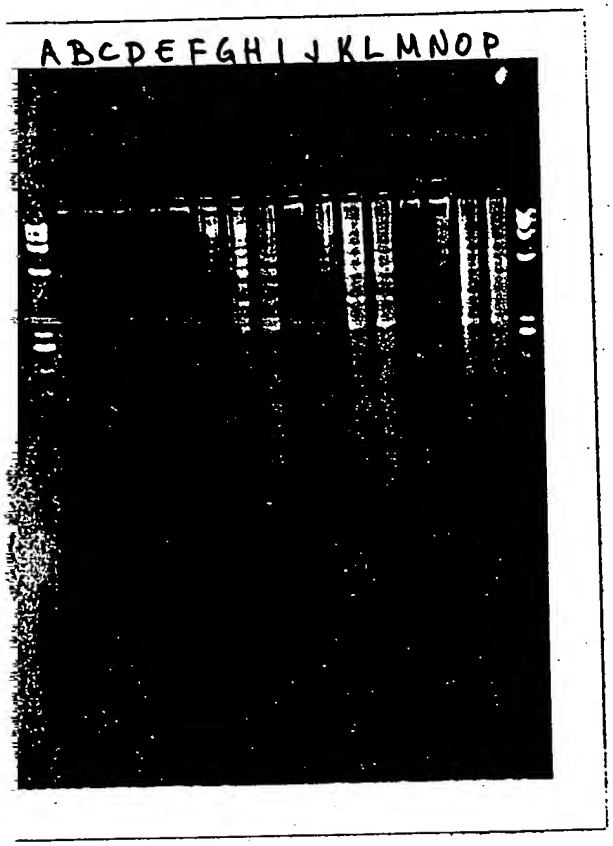
ck samples of Taq polymerase assay on gel and t if interesting.

R's described page 111. Load 5µl each sample onto % NuSieve / 0.5% agarose / 1x TBE → ON, 15V (~15hr)



15 cycles : : : :
20 25 30

A B C D	4µl	lot 1
E F G H	1	lot 2
I J K L	2	"
M N O P	4	"

Don't see anything with lot 1 polymerase. Lots of bands with the new stuff. Amount of between-band streaking seems to increase with amt. of enzyme added. No significant increase in overall flourescense 25 vs. 30 cycles even with 4µl lot 2 polymerase. May need to dilute out (<1µl) to reduce complexity and increase specificity.

This is worth blotting.

enature: ~200ml 0.5N NaOH, 1.5M NaCl → 45', RT

ithout neutralizing, transfer to Genatran with 20x SSPE → ON (~16hr)

inse filter in 20x SSPE and bake → 60' @ 80° in vacuum oven.
abel filter 2233:112

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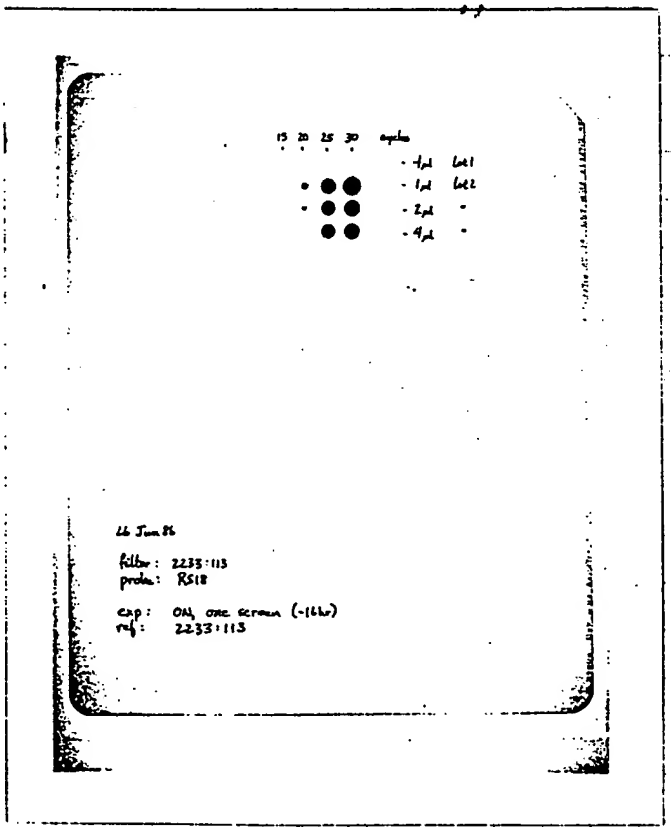
Prepare dot blot containing PCR samples from Taq polymerase assay described page 111.

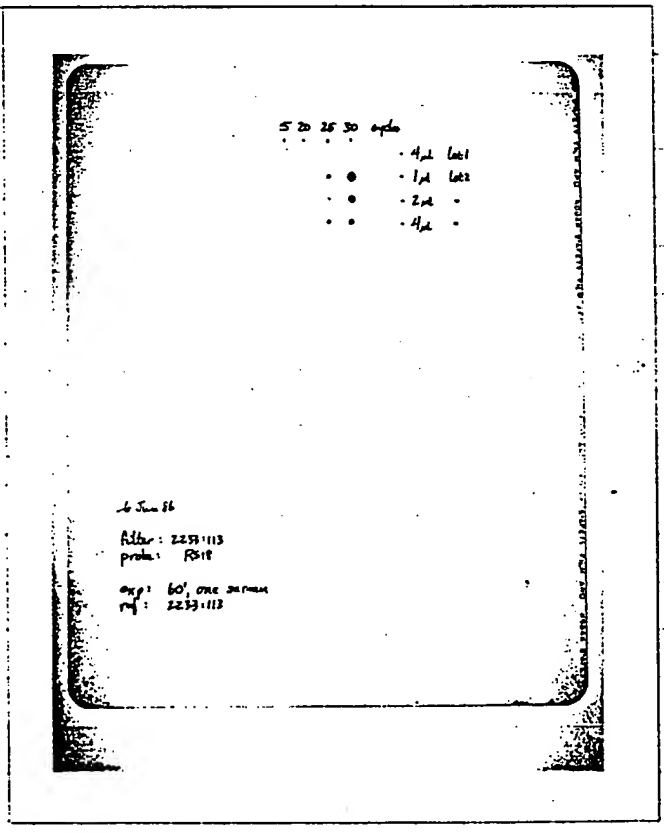
Apply 5µl each sample to filter: 5µl PCR sample
195µl 0.4N NaOH, 25mM EDTA
200µl

Rinse each well with 0.4 ml 20xSSPE, then entire filter in 20x.
Bake 1 hr @ 80°, in vacuum oven.
Label filter 2233:113

RS18 @ 0.057 pmol/µl, 0.5 pmol → 8.8µl (page 107)

Prehyb: 10ml 5xSSPE, 5x DET, 0.5% SDS → 15', 55°
Hyb: above + 0.5 pmol RS18 → 60', 55°
Wash: 3x 100 ml 2xSSPE, 0.1% SDS → 5-10', RT each
1x 100 ml 5xSSPE, 0.1% SDS → 5', 60°





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TITLE PCR: Taq Polymerase Assay (Lot 2) - II Book No.

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Repeat Taq pol assay essentially as described page 111 except with amounts of enzyme ≤ 1µl.

A B C D:	1 µl	(lot 2)		
E F G H:	1/2 µl	"	A, E, I, M, Q, U:	15 cycles
I J K L:	1/4 µl	"	B, F, J, N, R, V:	20 "
M N O P:	1/8 µl	"	C, G, K, O, S, W:	25 "
Q R S T:	1/16 µl	"	D, H, L, P, T, X:	30 "
U V W X:	1/32 µl	"		

Molt4 @ 100 µg/ml, PC03 and PC04 @ 5µM, dNTP @ 40mM, gelatin @

80µl	DNA	
80µl	10x salts	Divide into five 100µl and one 200µl
80µl	gelatin	samples and heat → 10', 98°
80µl	PC03	Cool to RT and to 200µl sample
80µl	PC04	add 2µl Taq pol (lot 2).
80µl	DMSO	Prepare five 2-fold 100µl serial
105µl	dNTP	dilutions in remaining 5 tubes.
200µl	H_2O	Transfer 100µl (range: 1µl to 1/16µl
800µl		Taq pol per tube)
		Sub-divide each further into four
		25µl samples and overlay with
		mineral oil (total: 24 tubes)

Subject to 14-29 cycles: | 2½' @ 98° | 3' @ 37° | ← new program 3' @ 37° instead of 5 min

After last cycle incubate additional 10' @ 60° to finish off final extension.

Extract mineral oil with $CHCl_3$. Store at -20°

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Hybridize Southern of Taq polymerase assay with RS18.

RS18 @ 0.057 pmol/μl, 0.5 pmol → 8.8μl (page 107)

Prehyb: 20ml 5XSSPE, 5XDET, 0.5% SDS → 45', 55°
Hyb: above + 0.5 pmol RS18 → 90', 55°
Wash: 3x100ml 2x SSPE, 0.1% SDS → ~5', RT each
1x100ml 5x SSPE, 0.1% SDS → ~15', ~~55~~ 60°

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Hyb: 2233:113, 117 with RS18

TITLE Dot Blot: Taq Pol Assay (Lot 2) - II

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Prepare dot blot containing PCR samples from Taq polymerase assay with ≤ 1 µl enzyme per reaction.

Samples described page 115.
Apply 5µl each sample to filter: 5µl PCR sample
195µl 0.4N NaOH, 25mM
200µl

Work-up as described page 113.
Label filter 2233:117

~~Hybridize RS18 to above filter along with a copy of 2233:113~~

Hybridize RS18 to filter above plus 2233:113 as positive control.

RS18 @ 0.057 pmol/µl, 0.5 pmol → 8.8µl (p.107)

Prehyb: 10ml 5xSSPE, 5x DET, 0.5% SDS → 15', 55° (both filters in same bo
Hyb: above + 0.5 pmol RS18 → 3hr, 55°
Wash: 3x 100ml 2xSSPE, 0.1% SDS → ~5', RT each
1x 100ml 5xSSPE, 0.1% SDS → 20', 60°

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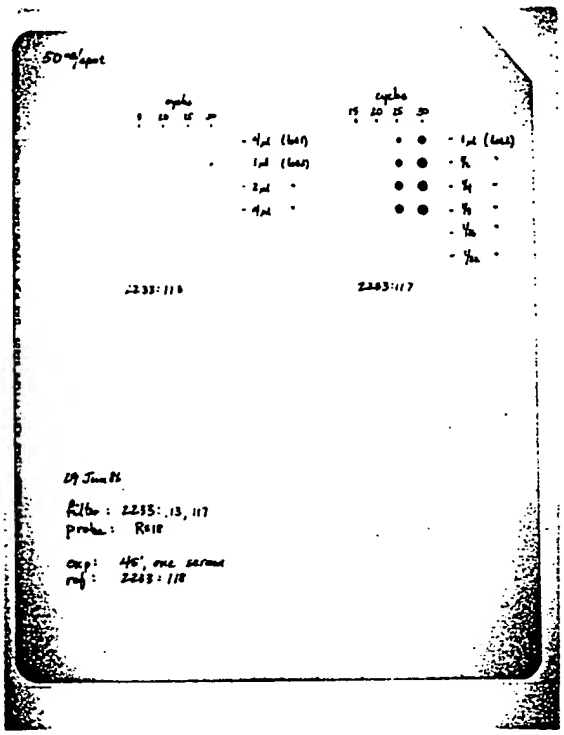

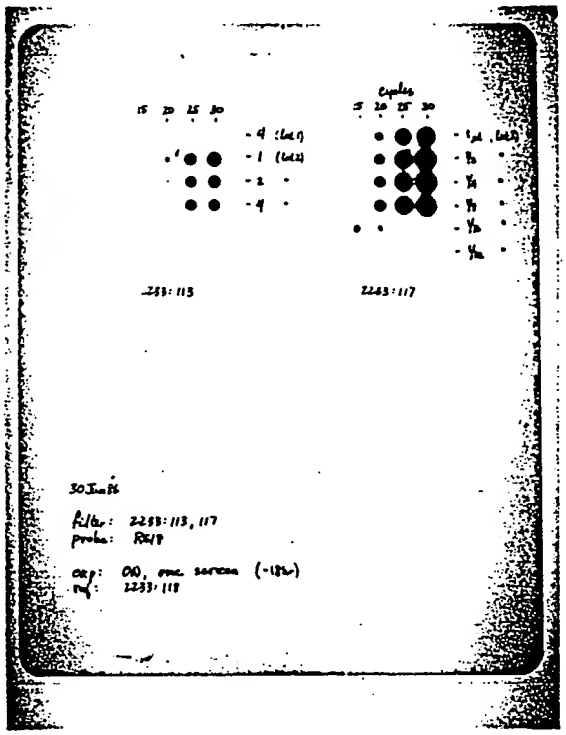

1/8 µl is optimal amount of enzyme (with lot 1). As before, there is an abrupt cut-off below optimal → no signal with 1/16 µl. Signal improves as amount added per PCR reaction is reduced from 4 µl to 1/8 µl.

Lower intensity of signals on 2233:113 relative to 2233:117 probably due to loss of DNA when former was stripped prior to re-hyb.

Not sure if signals in 1/16 µl samples after ON exposure are real. Possible that order of those spots was inverted somehow. But could also be artifact. Whatever it is, it is pretty insignificant when compared to 1/8 µl spots.

Clearly, the new PCR program (3' @ 37° vs 5' @ 37°) works just fine.

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